Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11145–11149, November 1995
Neurobiology

Layer-specific programs of development in neocortical projection neurons

(cerebral cortex/corticothalamic projection/thalamus/ferret/axonal growth)

FRANCISCO CLASCÁ*, ALESSANDRA ANGELUCCI, AND MRIGANKA SUR

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

Communicated by Richard Held, Massachusetts Institute of Technology, Cambridge, MA, August 21, 1995

ABSTRACT **How are long-range axonal projections from the cerebral cortex orchestrated during development? By using both passively and actively transported axonal tracers in fetal and postnatal ferrets, we have analyzed the development of projections from the cortex to a number of thalamic nuclei. We report that the projections of a cortical area to its corresponding thalamic nuclei follow highly cell-specific programs of development. Axons from cells in the deepest layers of the cerebral cortex (layer 6 and superficial subplate neurons) appear to grow very slowly and be delayed for several weeks in the cerebral white matter, reaching the thalamus over a protracted period. Neurons of layer 5, on the other hand, develop their projections much faster; despite being born after the neurons of deeper layers, layer 5 neurons are the first to extend their axons out of the cortical hemisphere and innervate the thalamus. Layer 5 projections are massive in the first postnatal weeks but may become partly eliminated later in development, being overtaken in number by layer 6 cells that constitute the major corticothalamic projection by adulthood. Layer 5 projections are area-specific from the outset and arise as collateral branches of axons directed to the brainstem and spinal cord. Our findings show that the early development of corticofugal connections is determined not by the sequence of cortical neurogenesis but by developmental programs specific for each type of projection neuron. In addition, they demonstrate that in most thalamic nuclei, layer 5 neurons (and not subplate or layer 6 neurons) establish the first descending projections from the cerebral cortex.**

The highly ordered connections of the mammalian neocortex are the substrate for its remarkable functional specificity. The mechanisms that coordinate the assembly of these connections during development remain to be elucidated. The order of neuronal birthdate of neocortical neurons has been widely considered a key determinant of the temporal sequence in which cortical connections develop (1–5). This is based on the evidence that neocortical neurons start growing their axons as soon as they reach the cortical plate or even earlier (2–7), as well as on the intuition that, because of the rapid expansion of the fetal brain, all neurons should elongate their axons as quickly as possible to lay down pathways while distances and navigational problems are at a minimum (2–5). There is no evidence, however, that all cortical neurons extend their axons rapidly or at the same pace. Indeed, evidence from certain peripheral sensory ganglia indicates that neurons that have to cover different distances to their targets have cell-specific programs that regulate the rate of their axonal growth (8, 9). We hypothesized that similar cell-specific programs of development, rather than the sequence of birthdates, might determine the timing by which neocortical neurons establish their connections.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

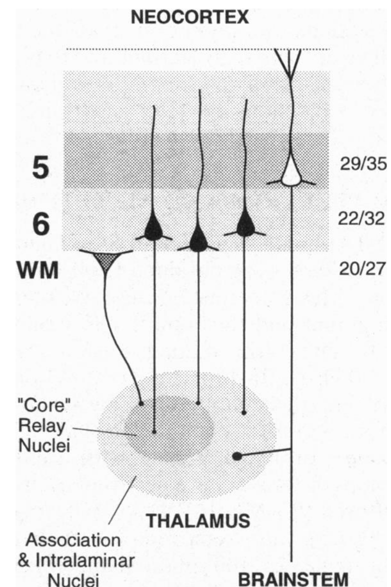

FIG. 1. Highly simplified scheme of projections to the thalamus from the adult neocortex (10–13). The embryonic range of ages (in days) when the neurons destined for each layer in the ferret (visual) cortex are generated (14) is indicated on the right. WM, white matter.

For this analysis, we chose to examine the corticothalamic projection, a massive efferent system of the cerebral cortex. Each neocortical area sends projections to a specific set of thalamic nuclei (Fig. 1). The neurons that give rise to this projection are born at different embryonic ages but extend their axons along the same pathway. Their somata are located in cortical layers 5 and 6 and in the most superficial part of the subcortical WM (10–12). The latter of these projection neurons are born first; these are a remnant of the cells of an embryonic layer of the cerebral cortex know as the subplate (SP) zone (1, 14, 15). Neurons in layers 6 and 5, in order, are born progressively later (1, 16, 17). Previous studies on the development of corticothalamic projections (2, 5, 7, 18–21) have focused on the projections from primary visual cortex (area 17) to the dorsal lateral geniculate nucleus (dLGN), the visual thalamic relay nucleus. However, this particular pathway represents only a subset of the general scheme of corticothalamic projections, since in most species the dLGN does not receive layer 5 projections from area 17 (10, 22–24). Moreover, layer 5 axons from area 17 directed to the midbrain run through and around the dLGN, compromising the interpre-

Abbreviations: CTB, cholera toxin subunit B; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; dLGN, dorsal lateral geniculate nucleus; FB, fast blue; E, embryonic day; IC, internal capsule; SP, subplate; WM, white matter.

*To whom reprint requests should be sent at the present address: Department of Morphology, Medical School, Autónoma University, Avenue Arzobispo Morcillo s/n, Madrid, 28029 Spain.

Table 1. Experimental animals analyzed in this study

	No. of animals											
Tracer, site	E31	E34	E37	E41	E42	E43	E44	E46	E50	E54	E58	E62
DiI, thalamus	2	2	6	1	12	2	2	8	11	9	8	4
FB/CTB, thalamus					2	10	6	8	6	3	4	3
CTB, cortex					6	3		2	2	1	1	2
DiI, WM and cortex			1	1	1			2	2	6	1	2

DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; FB, fast blue; CTB, cholera toxin subunit B.

tation of cortical labeling after injections of retrogradely transported axonal tracers into the dLGN (18, 19, 21, 25). Thus, in addition to the dLGN, we examined the development of cortical projections to a number of thalamic nuclei known to receive both layer 5 and 6 projections and to be free of other descending fibers of passage. Our findings demonstrate that corticothalamic projections develop according to programs specific for the cells of each cortical layer.

MATERIALS AND METHODS

Experimental Animals. Neonatal or fetal pigmented ferrets (*Mustela putorius furo*) were obtained from females mated in our colony. We chose ferrets because of their protracted cortical development and the remarkable immaturity of the cortex at birth. The 24-hr mating session was designated embryonic day 0 (E0). Birth occurred 40–43 days later. For consistency, we refer throughout this study to age in embryonic days, both before and after birth (Table 1).

Axonal Tracing in Fixed Tissue with Lipophylic Dyes. Anesthetized pups or fetuses were perfused transcardially with phosphate-buffered (0.1 M, pH 7.4) 4% (wt/vol) paraformaldehyde. Their brains were removed, and their thalami were implanted with single (25–100 μm in diameter) crystals of DiI at selected locations. We analyzed the period from E31 to E62 (Table 1). After E37, thalamic differentiation allowed selective implants in individual nuclei, namely, the lateral and medial geniculate nuclei, as well as the mediodorsal, ventromedial, ventroposterior, and centromedian-parafascicular nuclei. A further series of brains received similar DiI implants in the cerebral cortex or at various points [perireticular nucleus, internal capsule (IC), and cerebral WM] along the subcortical pathway to thalamus. To allow for dye diffusion, the brains were stored in phosphate-buffered 0.1% sodium azide for 3–10 weeks (E31–46) or 12–16 weeks (E50–62) at 27°C and then coronally sectioned at 150 μm with a Vibratome. We examined the labeling by using conventional and confocal epifluorescence microscopy on water-mounted sections. Dark-field illumination or bisbenzimide counterstain (0.005% in saline, 10 sec) of selected sections allowed precise localization of the labeling.

Retrograde Axonal Tracing *in Vivo*. Ferret pups were anesthetized by hypothermia or with ketamine (70 mg/kg) and received stereotaxic injections of FB [2% (wt/vol) in water; Sigma] or CTB (1%; List Biological Laboratories, Campbell, CA) in the thalamus or the cerebral cortex through glass micropipettes. CTB was iontophoresized (2 μA, 7 sec on/off, 2–10 min total), and FB (0.1–0.3 μl, in water) was pressure-injected by using a Picospritzer II (General Valve, Fairfield, NJ). The surgical wound was sutured, and the pups were returned to the mother. After 28–96 hr of survival, the pups were perfused as above. Age at perfusion was considered the age of the experiment. The brains were soaked in phosphate-buffered 30% (wt/vol) sucrose and freeze-sectioned at 40 μm. We found that in neonatal tissue, FB labeling fades rapidly; thus, we analyzed and photographed the FB labeling in phosphate-buffer-mounted sections immediately after cutting. CTB labeling was revealed immunohistochemically (unpublished data) and analyzed under bright- and dark-field optics. We discarded from analysis any case with contamination of the cortex or midbrain along the micropipette track. Although FB injections turned out to be larger and, hence, labeled many more neurons than CTB injections, both tracers gave similar results on the pattern of cell distribution. In addition, for comparison, three adult ferrets received similar CTB and FB stereotaxic deposits in the thalamus under ketamine (15 mg/kg) and xylazine (2.5 mg/kg) anesthesia and had their brains processed and analyzed as described above. To localize

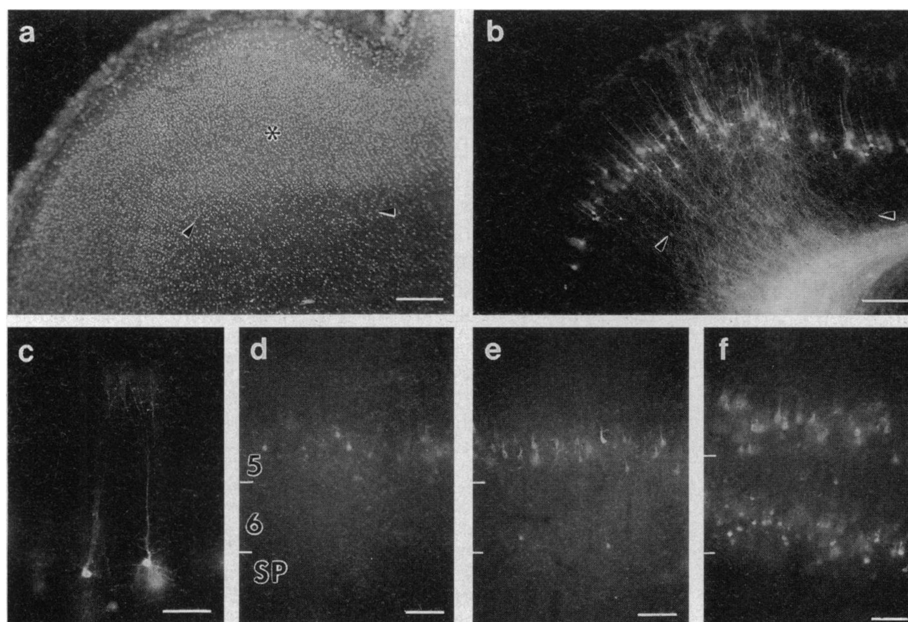

FIG. 2. Corticothalamic neurons retrogradely labeled by injections of fluorescent tracers in the thalamus. (*a*) A coronal section of the auditory cortex in an E50 ferret brain, stained with bisbenzimide to reveal the cellular layers of the cortex under epifluorescence illumination. Arrowheads indicate the border between layer 6 and the SP. At this age, layer 5 (asterisk) is already distinguishable by its lower cell density. (Bar = 250 μm.) (*b*) The same field as in *a* but viewed through epifluorescence filters to reveal DiI labeling, as a result of a DiI implant in the medial geniculate nucleus of the thalamus. (Bar = 250 μm.) (*c*) Higher-power view of the pyramidal neurons shown in *b*. (Bar = 100 μm.) (*d–f*) Corticothalamic neurons labeled in the sensorimotor cortex after FB injections in the ventrolateral and ventroposterior thalamic nuclei at E43 (*d*), E46 (*e*), and E54 (*f*). (Bars for *d–f* = 200 μm.)

brain structures, additional series of sections were Nissl-stained.

Anterograde Tracing *in Vivo*. CTB produces detailed anterograde labeling of fiber tracts and terminals but does not label the axons of retrogradely labeled cells (A.A. *et al.*, unpublished data). Thus, CTB was also injected in parietal or occipital cortex in a number of cases to anterogradely label corticothalamic axons.

RESULTS

Corticothalamic Development Revealed by Retrograde Transport of DiI. To label cortical cells projecting to the thalamus, we first implanted crystals of DiI in several thalamic nuclei of fixed fetal and postnatal brains (Table 1). From the earliest ages examined (E31), such implants labeled large numbers of thalamocortical axons extending up to the SP beneath the corresponding cortical zone. Before E46, however, retrogradely labeled neurons were either absent or very scarce. Such occasional neurons were invariably located in the deepest part of the SP zone, amid intensely stained thalamocortical axons, and were associated with labeling in radial glial cells. After E46, a large number of pyramidal neurons were labeled in cortical layer 5 (Fig. 2 *a–c*). On the other hand, neurons in upper SP or in layer 6 were not consistently labeled until E50–58; the precise timing depended on the cortical area examined.

Corticothalamic Development Revealed by Retrograde Tracers *in Vivo*. We suspected that the inconsistent neuronal and glial labeling observed at ages younger than E42 may have been due to DiI diffusion from labeled thalamocortical fibers (7, 26). Thus, we carried out a parallel series of experiments with sensitive tracers that are axonally transported *in vivo*. Microinjections of FB or CTB in the thalamus at the earliest age analyzed (E42) labeled some scattered neurons in the upper half of the cortical plate but none in SP or layer 6. A dramatic change in the pattern of labeling occurred after injections in E43–44 animals: many pyramidal-shaped cells, forming a continuous band, were labeled in the upper half of the cortical plate (Figs. 2 *d* and *e* and 3). Again, no cell was labeled in SP or layer 6. The band of pyramidal cells was labeled throughout the period examined (up to E62) and was identified cytoarchitectonically as situated in layer 5 (Figs. 2 *d–f* and 3). In sharp contrast to the abrupt and nearly simultaneous appearance of layer 5 projections from various cortical areas (Fig. 3 *A* and *B*), labeling in upper SP and layer 6 neurons progressively increased in number and extent after injections at older ages. In each area, labeling appeared first in neurons situated immediately below the inner border of the cortical plate (in the upper SP), some of them displaying inverted pyramidal morphology. Cells in successively more superficial parts of layer 6 became labeled after injections in the following days or weeks, until the full adult complement was reached. These cells were smaller than the pyramidal cells in layer 5 (Fig. 2). In addition, FB and CTB experiments revealed a pronounced gradient across the cerebral hemisphere: in dorsal/posterior areas (Fig. 3*A*), labeling in upper SP and layer 6 cells appeared several days later than in more anterior/ventral areas (Fig. 3*B*) and took longer to reach its full complement.

Collateral Projections of Layer 5 Neurons. In ferrets, axons of layer 5 neurons have extended to the lower brainstem by E41 (27). When we injected CTB in the cortex during the period in which only layer 5 projections are present (between E44 and E50; Fig. 3*A*), this tracer anterogradely labeled collateral

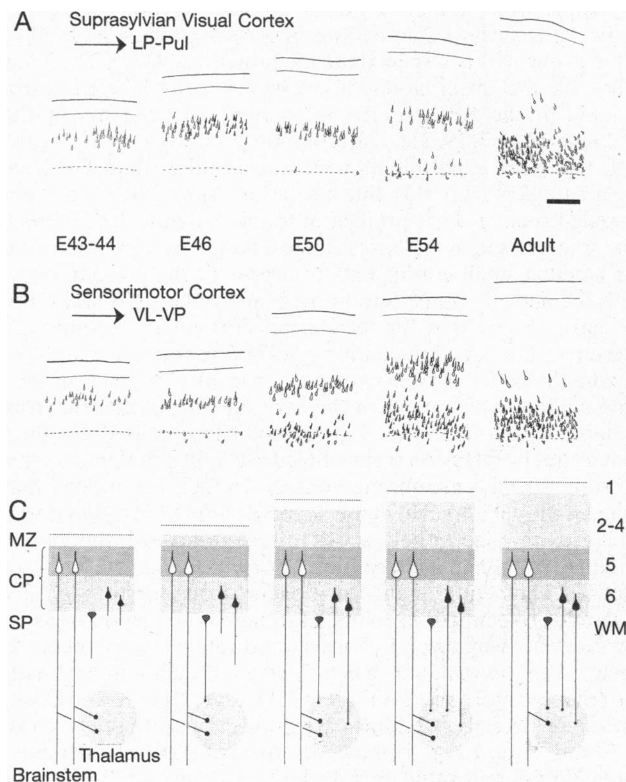

FIG. 3. Corticothalamic projections from two regions of the cortical hemisphere revealed by retrograde labeling after FB injections in the thalamus at different ages. (*A*) Labeling in posterior dorsal cortex (lateral suprasylvian visual cortex) after injections in the thalamus (lateral posterior-pulvinar nucleus, LP-Pul). The adult is a sample from posterior lateral suprasylvian cortex, after an injection in the same thalamic nucleus. (Bar = 0.5 mm.) (*B*) Labeling in anterior ventral cortex (lateral sensorimotor cortex) after injections in the ventrolateral-ventroposterior thalamic nuclei (VL-VP). The adult sample is from lateral primary somatosensory cortex after an injection in the ventroposterior nucleus. In each case, as in *A*, the samples are taken from the most heavily labeled region in the cortex. (*C*) Schematic interpretation of development of corticothalamic projections. Compare with Fig. 1. Figurines are matched to the sequence shown in *B*. MZ, marginal zone; CP, cortical plate.

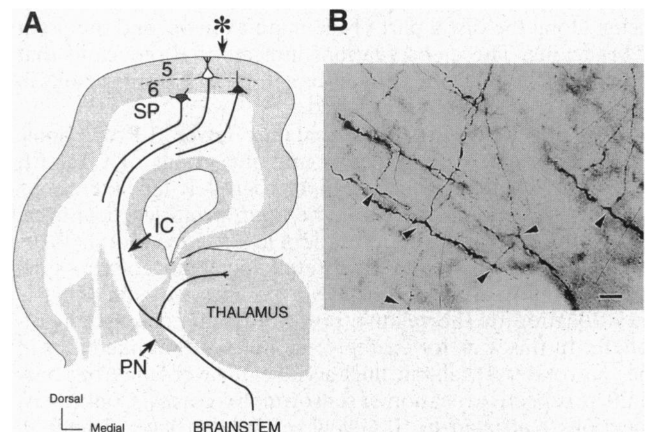

FIG. 4. (*A*) Schematic diagram illustrating the relative position of the various populations of developing corticothalamic axons arising from neurons in dorsal cortex early in development (see text for details). The figure shows that the axon of a layer 5 neuron has traversed the IC and grown a collateral branch toward the thalamus. The branch has arisen at a point where the main axon traverses the perireticular nucleus (PN). (*B*) High-power view of descending cortical axons labeled anterogradely by an injection of CTB in dorsal parietal cortex (at a point equivalent to that indicated with an asterisk in *A*) in an E50 ferret. The image is taken at the level of the perireticular nucleus. The main axon trunks run obliquely from the left to the bottom right of the image. Collateral branches arise from the axons (arrowheads) and continue toward the thalamus, which is situated toward the upper right. (Bar = 10 μm.)

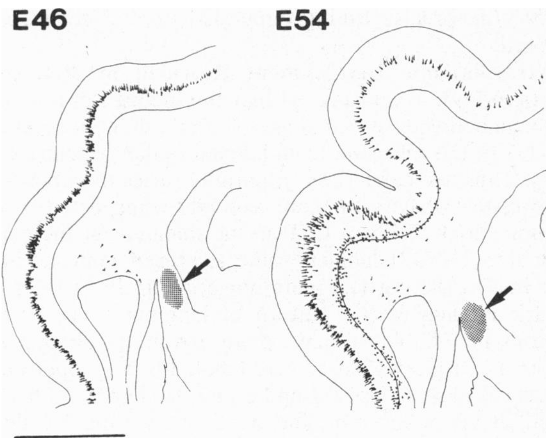

FIG. 5. Camera-lucida drawings showing the neurons in the cerebral cortex labeled by implanting a DiI crystal in the internal capsule (arrow) at different ages. The orientation of these drawings is the same as Fig. 4*A*, and the DiI crystal is approximately located at the arrow marked IC in Fig. 4*A*. At E46, only layer 5 cells are labeled from the implant. The absence of labeled cells along the inner border of the cortical plate (corresponding to layer 6 and upper SP; see Fig. 3) indicates that none of their axons have yet extended to the site of the implant. By E54, not only layer 5 but also a substantial number of upper SP and layer 6 cells in the lateral part of the hemisphere are labeled by an implant in approximately the same site. Note that cells deep below the cortical plate (deep SP cells) are only labeled close to the injection site at E46 or E54, suggesting that these cells extend their axons only a short distance into the internal capsule. (Bar = 1 mm.)

branches that emerged perpendicularly from corticofugal axons that continued into the cerebral peduncle (Fig. 4). Nissl counterstaining revealed that the branches occurred at the point where the axons traversed the perireticular nucleus. Collaterals were the rule: in serial reconstructions, fibers entering the thalamus could be always traced down to a branch point from a thicker trunk directed toward subthalamic levels. Moreover, after injections of FB or DiI in thalamic nuclei at these ages, we observed massive fiber labeling in the pyramidal or corticopontine tracts, presumably due to anterograde diffusion along the distal part of the main axon beyond the point of branching. These observations indicate that the axons that give rise to the thalamic collaterals extend to distant regions in the brainstem and/or spinal cord.

Areal Specificity of Corticothalamic Layer 5 Projections. When DiI deposits or thalamic microinjections of CTB/FB were limited to particular nuclei, retrogradely labeled layer 5 cells were largely restricted to the appropriate cortical area. Although cytoarchitectonic borders are not evident at these early ages, the dimples that foretell the main cortical sulci appear around E46 (Fig. 5). At younger ages, we estimated the areal location by the relative position on the cerebral hemisphere. In this way, for example, we noticed that injections in the ventrolateral thalamic nucleus labeled layer 5 neurons only in the prospective location of sensorimotor cortex. Conversely, injections restricted to dLGN never labeled layer 5 cells in dorsal occipital cortex (presumptive area 17; ref. 27), which in the adult also does not send layer 5 projections to dLGN; however, they labeled layer 5 cells in more lateral and anterior occipital cortex (presumptive areas 18 and 19), which in the adult do send layer 5 projections to dLGN (10, 22–24).

Delayed Growth of Layer 6/Upper SP Projections. Retrograde tracing from tracer deposits in the thalamus did not resolve whether axons from layer 6 and upper SP neurons elongate slowly toward the thalamus or, alternatively, arrive rapidly near the thalamus but "wait" before invading their target nuclei (3, 5). We tested these possibilities by implanting DiI crystals at different points along the pathway from the cerebral cortex to the thalamus (to impregnate all axons present in that part of the pathway at a given age) and subsequently examining the pattern of retrograde labeling in the cortex. Implants in IC started labeling large numbers of layer 5 neurons as early as E40. However, upper SP and layer 6 neurons only became labeled after similar DiI implants at much older ages (E50–58), about the time when they had been labeled after thalamic injections of FB or CTB (Figs. 3*A* and 5). Thus axons of upper SP and layer 6 cells appear to elongate slowly in the WM.

DISCUSSION

By using a variety of sensitive axonal tracers *in vivo* and in fixed tissue and selective application in specific thalamic or cortical locations, we have revealed striking differences in the timing of target innervation and rate of elongation of the various populations of corticothalamic axons. Axons of layer 5 neurons elongate rapidly and enter the thalamus first, whereas those of upper SP/layer 6 neurons grow slowly and enter the thalamus over a protracted period, subsequent to layer 5 axons.

The sequence of cortical neurogenesis has been widely assumed to dictate the development of its projections (1–5, 28). Anterograde axon tracing experiments using DiI have shown that axons of early cortical neurons, presumably SP cells, reach the internal capsule very early in development (2–4, 6, 7, 19, 21). It was suggested that SP axons elongate subsequently to the appropriate thalamic nuclei, pioneering the corticothalamic pathway; later populations of cortical axons would follow these axons to reach their thalamic targets (2, 4, 21). This view relies on the observation of occasional cells labeled retrogradely in the SP at early ages after DiI implants in the thalamus (2, 3, 7, 19, 21). Anterograde (3, 5, 18) and retrograde (20) transport experiments with tracers other than DiI have been at odds with this interpretation, since they have not revealed such an early projection to the thalamus (see below). The tracers used in the latter studies, however, were not suited for labeling small contingents of axons. In the present study, with the actively transported retrograde tracers FB and CTB, we have shown that, in ferrets, no cortical projections are present in the dorsal thalamic nuclei before the arrival of layer 5 axons. Since DiI is known to undergo transneuronal transport (26), a likely explanation for the early labeling of SP cells from thalamic implants is that DiI stained these cells (as it does radial glia) by diffusion from labeled ascending thalamic axons, possibly through membrane contact. In fact, we noticed that some of the cells labeled in the deep SP with DiI in our material had growth-cone-tipped axons directed toward the dorsal midline of the hemisphere instead of the thalamus (data not shown). The requirement for pioneer axons for the corticothalamic projection is itself unclear: there is no obvious reason, for example, why layer 5 axons should rely on SP pioneers to navigate to the thalamus when they can do without such aids for reaching more distant targets (21). Our data are consistent with some SP cells extending early projections into the IC (refs. 2, 3, and 21 and Fig. 5); however, axons of SP cells unequivocally do not enter thalamic nuclei at early ages, before axons of layer 5 cells.

After injections of retrograde tracers in the dLGN, previous studies (19–21, 25) have reported labeling in layer 5 cortical neurons (presumably outside area 17) at about the same age at which the first labeling in layer 6 appeared. Because layer 5 axons directed to the ventral lateral geniculate nucleus and midbrain run through and around dLGN, the significance of this labeling was unclear (19–21, 25). Our injections in thalamic nuclei, such as the dorsomedial, ventromedial, ventral lateral, and ventral posterior nuclei that are free of corticofugal axons passing toward the brainstem, show unequivocally that layer 5 neurons are the first to project to the thalamus.

In agreement with recent findings in adult cats and rats (12, 13), our data show that corticothalamic layer 5 projections are

collateral branches of projections directed to other subcortical targets. Collaterals grow once layer 5 axons have extended far below the thalamus (27), sprouting from a region (the perireticular nucleus) where the axons from all cortical areas are closely adjacent. This may explain why the layer 5 projections develop simultaneously in the various areas examined and suggests that some signal acting locally may trigger the development of the collaterals. The perireticular nucleus is a partly transient cell group that itself sends an early topographic projection into the thalamus (29). It is tempting to speculate that the perireticular nucleus may guide the layer 5 collaterals in their targeting (30); however, other collateral projections from layer 5 axons are also precise from the onset (31). Our injections in the dLGN labeled layer 5 cells in areas 18 and 19 but not in presumptive area 17. An interpretation consistent with this observation is that areal boundaries in cortex are demarcated, at least broadly, by E43–44. This argues for very early specification of certain features of neocortical areas (32), for at this age specific thalamocortical axons have not invaded the occipital cortical plate (25).

Projections from layer 5 remain massive by the end of the third postnatal week. By adulthood, however, they have become drastically reduced (Fig. 3). Our interpretation (Fig. 3*C*) is that some collaterals are removed at later postnatal ages. While the mechanisms responsible for removal of these and other collaterals from corticofugal axons are unknown (33), it is striking that, in the thalamus, projections that have apparently reached their appropriate area-specific target nuclei are removed.

Previous studies with anterograde tracers injected into visual cortex noted that labeling in the dLGN appears days after cortical projections have extended to the brainstem (3, 5, 18). Since projections to the brainstem arise only from layer 5 cells, it was suggested that upper SP/layer 6 axons may be delayed in the periphery of the LGN before entering the nucleus. In ferrets, by E36, upper SP/layer 6 cells have completed their migration even in those regions where neocortical neurogenesis is more delayed (14). From our DiI implants in the WM, it is clear that most of these cells do not extend their axons far from their area of origin for at least 2–3 weeks after becoming postmigratory. Layer 5 axons, on the other hand, start crossing the internal capsule, the gateway out of the cerebral hemisphere, shortly after becoming postmigratory (14). Fast axonal growth may allow layer 5 cells to reach caudal segments of the neuraxis early on, in spite of their late neurogenesis. Axons of upper SP and layer 6 cells elongate slowly and display similar developmental programs. The marked temporal scatter across areas and layers in the arrival of these axons to their thalamic targets is what should be expected if the axons grow at roughly the same rate, given the different proximity of the various areas to the thalamus, the neurogenetic gradient between areas and layers (1, 14–17), and the fast growth of the fetal brain.

The slow axonal growth of layer 6/upper SP neurons is reminiscent of neurons in proximal chicken sensory ganglia (8, 9), whose axons are intrinsically specified to grow slower than neurons from more distal ganglia. A consequence of such variations in rates of growth is synchronization in the arrival of axons at their targets. From this perspective, the slow growth of upper SP/layer 6 cells might relate to the late development of distal dendritic segments of thalamic neurons (34), where these axons synapse (12, 13, 35, 36). Regardless of developmental role, our data indicate clear cell-specific differences among programs of development of projection neurons in different layers of the ferret neocortex. These programs could be specified during laminar fate determination (37). Alternatively, different programs may be the result of dynamic interactions of layer 5 and layer 6/SP axons with other axonal systems or with different substrates in the developing white matter.

We thank S. Kuffler for technical help. This work was supported by grants from the National Institutes of Health and the March of Dimes. F.C. is the recipient of a postdoctoral fellowship from the Fogarty International Center of the National Institutes of Health.

1. Bayer, S. A. & Altman, J. (1991) *Neocortical Development* (Raven, New York).
2. McConnell, S. K., Ghosh, A. & Shatz, C. J. (1989) *Science* **245,** 978–982.
3. McConnell, S. K., Ghosh, A. & Shatz, C. J. (1994) *J. Neurosci.* **14,** 1892–1907.
4. Blakemore, C. & Molnar, Z. (1990) *Cold Spring Harbor Symp. Quant. Biol.* **55,** 491–504.
5. Shatz, C. J. & Rakic, P. (1981) *J. Comp. Neurol.* **196,** 287–307.
6. Erzurumlu, R. S. & Jhaveri, S. (1992) *Cereb. Cortex* **2,** 336–352.
7. Johnson, K. & Casagrande, V. A. (1993) *Visual Neurosci.* **10,** 117–130.
8. Davies, A. M. (1989) *Nature (London)* **337,** 553–555.
9. Davies, A. M. (1994) *Trends Neurosci.* **17,** 195–199.
10. Jones, E. G. (1985) *The Thalamus* (Plenum, New York).
11. Velayos, J. L., Casas-Puig, R. & Reinoso-Suárez, F. (1993) in *Thalamic Networks for Relay and Modulation*, eds. Minciacchi, D., Molinari, M., Macchi, G. & Jones, E. G. (Pergamon, Oxford), pp. 185–195.
12. Bourassa, J., Pinault, D. & Deschenes, M. (1995) *Eur. J. Neurosci.* **7,** 19–30.
13. Ojima, H. (1994) *Cereb. Cortex* **4,** 646–665.
14. Jackson, C. A., Peduzzi, J. D. & Hickey, T. L. (1989) *J. Neurosci.* **9,** 1242–1253.
15. Luskin, M. B. & Shatz, C. J. (1985) *J. Neurosci.* **5,** 1062–1075.
16. Rakic, P. (1974) *Science* **183,** 425–427.
17. Luskin, M. B. & Shatz, C. J. (1985) *J. Comp. Neurol.* **242,** 611–631.
18. Distel, H. & Holländer, H. (1980) *J. Comp. Neurol.* **192,** 505–518.
19. Miller, B., Chou, L. & Finlay, B. L. (1993) *J. Comp. Neurol.* **335,** 16–41.
20. Sheng, X.-M., Marotte, L. R. & Mark, R. F. (1991) *J. Comp. Neurol.* **307,** 17–38.
21. De Carlos, J. A. & O'Leary, D. D. M. (1992) *J. Neurosci.* **12,** 1194–1211.
22. Gilbert, C. & Kelly, J. P. (1975) *J. Comp. Neurol.* **163,** 81–106.
23. Lund, J. S., Lund, D. R., Hendrickson, A. E., Bunt, A. A. & Fuchs, A. F. (1975) *J. Comp. Neurol.* **164,** 297–304.
24. Hendrickson, A. E., Wilson, J. R. & Ogren, M. P. (1978) *J. Comp. Neurol.* **182,** 123–136.
25. Ghosh, A. & Shatz, C. J. (1992) *J. Neurosci.* **12,** 39–55.
26. Godement, P., Vanselow, J., Thanos, S. & Bonhoeffer, F. (1987) *Development (Cambridge, U.K.)* **101,** 697–713.
27. Meissirel, C., Dehay, C. & Kennedy, H. (1993) *J. Comp. Neurol.* **338,** 193–213.
28. Gottlieb, D. I. & Cowan, W. M. (1972) *Brain Res.* **41,** 452–456.
29. Mitrofanis, J. & Baker, G. E. (1993) *J. Comp. Neurol.* **338,** 575–587.
30. Mitrofanis, J. & Guillery, R. W. (1993) *Trends Neurosci.* **6,** 240–245.
31. Kuang, R. Z. & Kalil, K. (1994) *J. Comp. Neurol.* **344,** 270–282.
32. Rakic, P. (1988) *Science* **241,** 170–176.
33. Stanfield, B. B. & O'Leary, D. D. M. (1985) *J. Comp. Neurol.* **238,** 236–248.
34. Dalva, M. M., Ghosh, A. & Shatz, C. J. (1994) *J. Neurosci.* **14,** 3588–3602.
35. Hogland, P. V., Wouterlood, F. G., Welker, E. & Van der Loos, H. (1991) *Exp. Brain Res.* **87,** 159–172.
36. Schwartz, M. L., Dekker, J. J. & Goldman-Rakic, P. S. (1991) *J. Comp. Neurol.* **309,** 289–304.
37. McConnell, S. K. (1988) *J. Neurosci.* **8,** 945–974.